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Prognostic Significance of Immunohistochemical Micrometastases in Node Negative Gastric Cancer Patients

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Background and Objectives: The purpose of this study is to examine the prognostic significance of immunohistochemical (IHC) evidence of lymph node (LN) metastases in histologic node negative gastric cancer patients.
Methods: Retrospective review from 1981 to 1998 revealed 25 patients resected for T1-4N0M0 gastric and gastroesophageal (GE) junction adenocarcinoma. All cases were reviewed and histopathologic parameters were defined for each primary tumor. All LNs underwent IHC analysis with the epithelial marker CAM 5.2. Data are reported as median (range).
Results: The median number of LN resected was 7 (range 1–33). The median follow-up time was 25 months (range 4–195) with an overall 5-year survival rate of 55%. For patients with IHC evidence of LN micrometastasis (n = 9), the 5-year survival rate was significantly decreased (35%) compared to a 66% 5-year survival rate for IHC negative patients (n = 16, $P = 0.05$).
Conclusions: The presence of IHC-detected LN micrometastases correlates with worse prognosis for patients with histologic node negative gastric cancer. IHC may be a useful additional staging modality in this subset of patients.
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KEY WORDS: gastric cancer; immunohistochemical; prognostic markers

INTRODUCTION

For patients with gastric adenocarcinoma, the presence of lymph node metastasis has consistently been shown to be an independent prognostic indicator [1,2]. Although patients with node positive gastric cancer have a significantly worse 5-year survival rate compared to patients without evidence of nodal metastases, a subset of patients with node negative gastric cancer will die of recurrent disease. Therefore, additional markers would be helpful in identifying patients at risk for recurrence.

Detection of metastatic disease in resected lymph nodes is routinely performed by evaluating hematoxylin and eosin (H&E) stained histologic sections using light microscopy. Considered the standard of care, the sensitivity of H&E evaluation is limited by the extent or number of cancer cells in a lymph node. Recent techniques using immunohistochemical (IHC) and molecular biologic methodologies have made it possible to detect micrometastatic disease not evident by routine H&E evaluation. Some authors [3,4] suggest that IHC detection of tumor cells in lymph nodes or bone marrow in patients with colorectal and breast cancer represents a marker of risk of recurrence. The significance of this "ultrastaging" is that detection of micrometastatic disease may offer additional prognostic information and/or offer a marker

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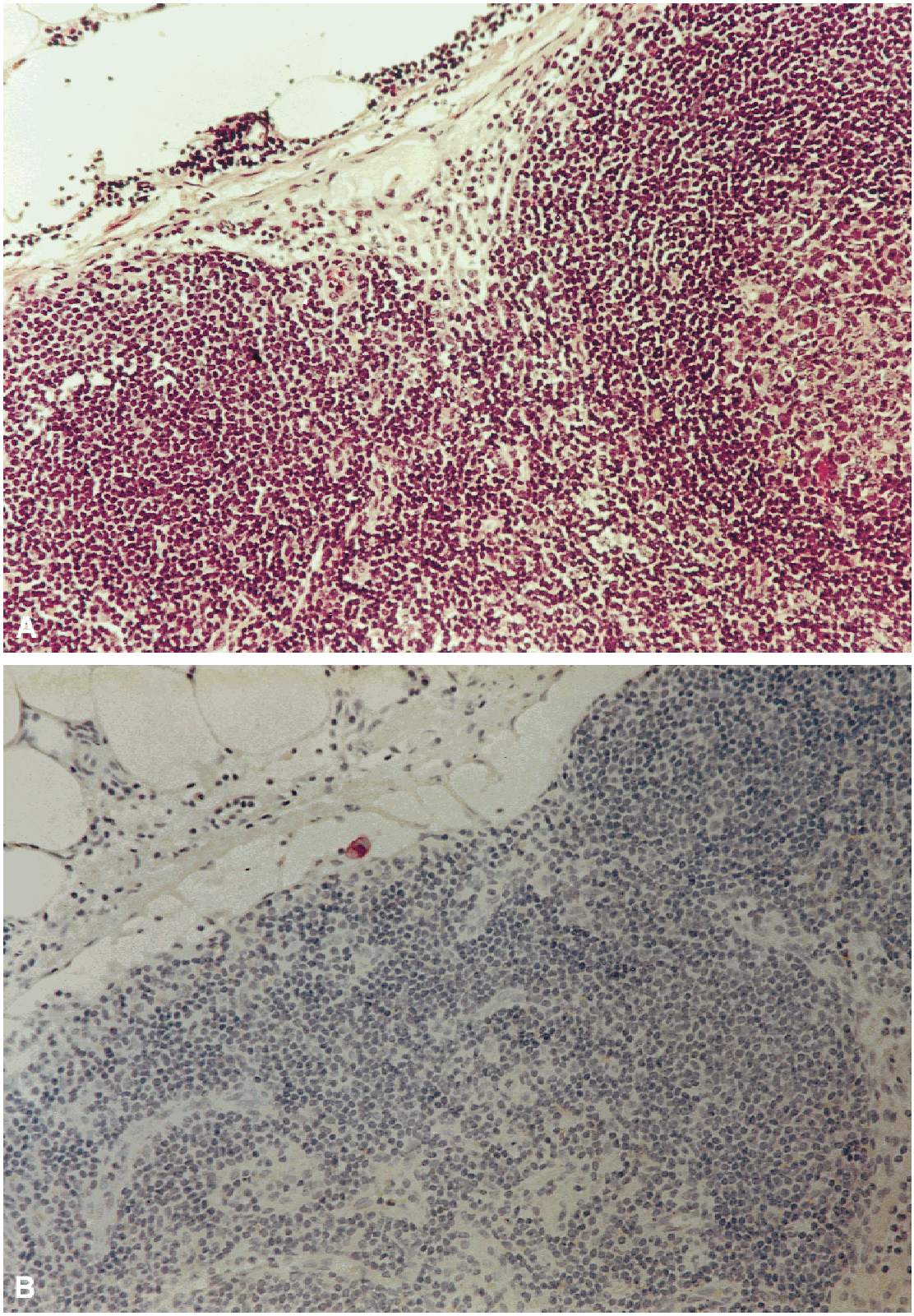

Figure 1.

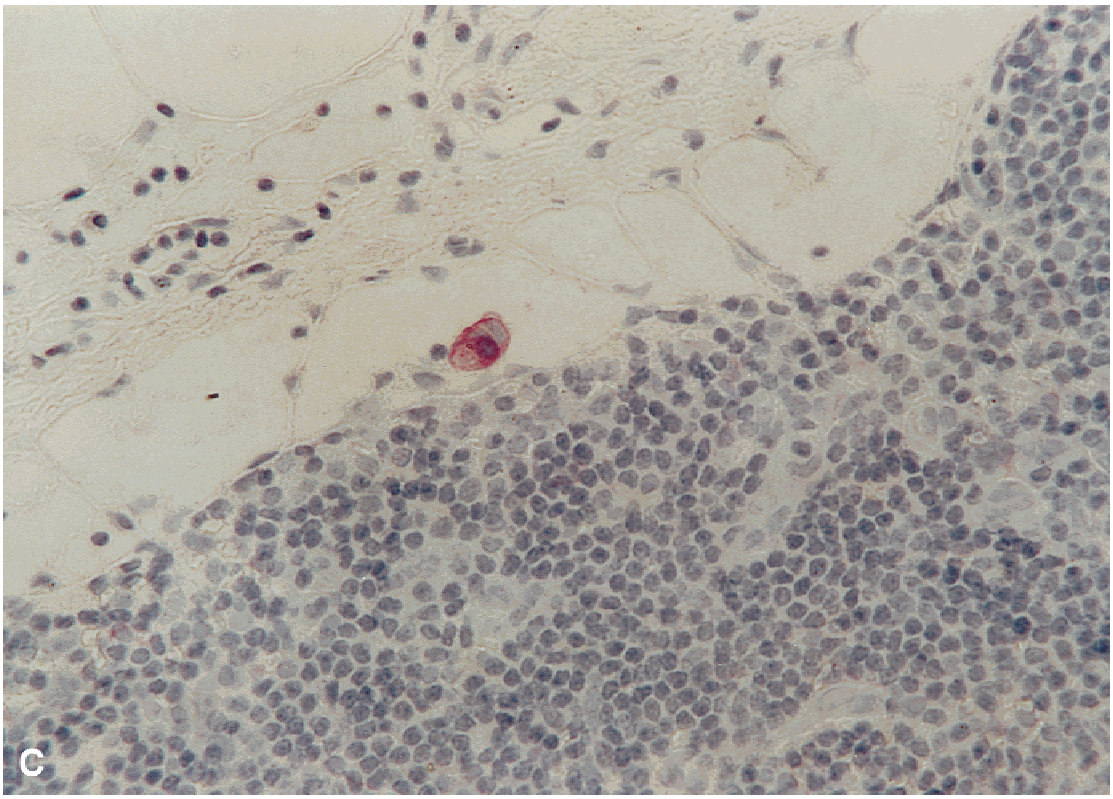

Fig. 1. An example of an H&E negative/IHC positive lymph node. **A:** H&E staining does not reveal the presence of micrometastatic disease. **B:** Photomicrograph of the same lymph node demonstrating cytokeratin staining with CAM 5.2 of metastatic disease. **C:** Higher magnification of the cytokeratin positive cell.

of disease that alters treatment. The purpose of this study is to determine the prognostic significance of IHC evidence of lymph node metastases in histologic (H&E) node negative gastric cancer patients.

MATERIALS AND METHODS

Retrospective review of the cancer registries at UMDNJ-New Jersey Medical School and East Orange Veterans Administration Medical Center from 1981 to 1998 revealed 29 patients resected for T1-4N0M0 gastric and gastroesophageal junction adenocarcinoma. A single pathologist at each institution reviewed all cases and histopathologic parameters were defined for each primary tumor. Pathologic review of the 29 cases identified four patients with previously missed H&E evidence of lymph node metastases. These patients were excluded from analysis.

All specimens were formalin fixed and paraffin embedded. One section from each lymph node was stained with H&E. An additional serial section was stained for the epithelial marker, CAM 5.2 (Becton Dickinson, San Jose, CA). Detection was performed by the phosphatase-anti-alkaline phosphatase technique. Data are reported as median (range). Survival was determined by the method of Kaplan-Meier and significance by the log-rank test. Statistical significance was defined as $P < 0.05$.

RESULTS

Over a 17-year period, 25 patients were identified as having histologic node negative gastric cancer. The median age of the entire group was 65 (43–78). A male-to-female ratio of 23:2 reflects a study population based at a VA hospital. Pathologic parameters are summarized in Table I. A total of 226 lymph nodes were examined, 24 (11%) of which were IHC positive in nine (36%) patients. The median number of lymph nodes examined per patient was 7 (range 1–33). Of the 25 patients, 20 had more than four nodes per specimen.

The median follow-up was 25 months (range 4–195). The actuarial 5-year survival rate for the entire cohort was 55%. The presence of lymphovascular invasion of the primary tumor ($n = 4$) was associated with a worse 5-year survival (25%), compared with patients without this feature (63%, $P = 0.01$). Lauren's classification was also identified as an important prognostic factor. Patients with intestinal or mixed-type histology ($n = 17$) had a 5-year survival rate of 81%, compared with those with the diffuse type ($n = 8$), who had a 19% 5-year survival rate ($P = 0.05$). Diffuse-type histology and grade correlated with the presence of lymph node micrometastases ($P < 0.01$, chi square).

For patients with IHC evidence of lymph node micro-

TABLE I. Clinical and Pathologic Features

Age (range)	64 (43–78)
Gender (M:F)	23:2
Median (range) of lymph nodes removed	7 (1–33)
Grade	
Well	4
Moderate	10
Poor	11
T stage	
T1	7
T2	8
T3	6
T4	4
Lauren classification	
Intestinal	16
Diffuse	7
Mixed	2
Lymphovascular invasion	
Yes	4
No	21
IHC	
Positive	9
Negative	16

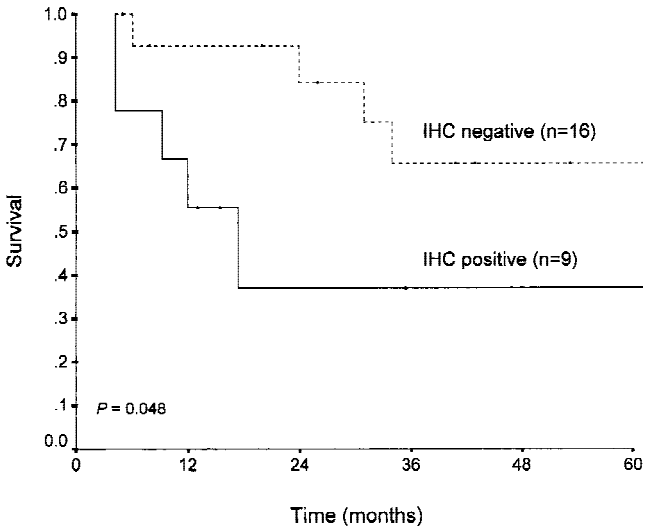

Fig. 2. For patients with IHC evidence of lymph node micrometastasis (n = 9), the 5-year survival rate was significantly decreased, i.e., 35% (median survival 17 months) compared to a 66% 5-year survival rate (median survival not reached) for IHC negative patients (n = 16, $P = 0.048$)

metastasis (n = 9), the 5-year survival rate was significantly decreased, i.e., 35% (median survival 17 months, 95% confidence interval 7–28 months). The 5-year survival rate was 66% (median survival not reached, 95% confidence interval 6–121 months) for IHC negative patients (n = 16, $P = 0.048$; Figs. 1,2).

DISCUSSION

Lymph node metastasis identified by traditional histopathologic examination is one of the most important prognostic factors for patients with gastric adenocarcinoma. The prognostic impact of lymph node metastasis by H&E evaluation can be demonstrated by significant differences in recurrence rates and survival [1,2]. The clinical significance of lymph node ultrastaging for histologically node negative gastric cancer is less well documented. Recent technologies using monoclonal antibodies against epithelial cell markers or tumor-associated antigens can detect tumor cells in tissues that are ostensibly free of disease by routine H&E examination. However, for IHC staging of lymph nodes to be relevant, the detection of micrometastatic disease should provide prognostic information above and beyond the standard clinicopathologic factors and/or should identify a subset of patients who may benefit from adjuvant therapy or close surveillance.

In this study, we demonstrate that the presence of IHC-detected metastases provides additional prognostic information in the select group of patients who have no evidence of lymph node disease by H&E evaluation. Some authors have suggested that IHC staging may be a useful staging technique for all stages of gastric cancer patients. Ishida et al. [5] studied 2,446 lymph nodes in 109 cases of stage I–IV gastric cancer with H&E and cytokeratin IHC staining. They found that whereas 230 (9.4%) lymph nodes were positive by H&E staining, an additional 201 lymph nodes (17.6%) were H&E negative, but IHC positive. Of the 109 cases, 11 patients were staged as histologically (H&E) negative, but IHC positive. Although the authors did not compare survival by IHC status for the H&E node negative cohort, they did report that IHC positivity predicted outcome for stage II patients. Similar to our results, they also demonstrated a correlation between IHC lymph node positivity and diffuse-type histology. In a similar study, Izbicki et al. [6] reported on IHC examination of both lymph node and bone marrow in patients with resectable esophageal cancer (n = 68). Interestingly, none of the 12 patients who were both H&E and IHC node negative had tumor recurrences. In contrast, patients with H&E negative but IHC positive cells in their lymph nodes had similar outcomes to those patients with H&E-proven lymph node metastases. By multivariate analysis, the presence of lymph node micrometastases was an independent risk factor for relapse-free and overall survival. This was true even for patients with H&E-proven disease. The presence of micrometastatic tumor cells in bone marrow had no additional prognostic value. In a review of 100 patients with early stage gastric cancer, Siewert et al. [7] demonstrated a high frequency of micrometastatic involvement of lymph nodes, which also correlated with both T stage and histology (diffuse vs. intestinal type). Micrometastatic disease, which they defined as the presence of three or more tumor cells in more than 10% of the resected lymph nodes (n = 10), was a significant pre-

dictor of survival compared to those with ≤10% involvement (n = 52). Micrometastatic disease was also an independent predictor of survival by multivariate analysis.

The presence of IHC positive cells in H&E negative lymph nodes has been used to support the argument for extended lymphadenectomy for gastric cancer. The rationale is based on the belief that removal of the micrometastatic disease will prevent further systemic metastasis. Interestingly, extended lymphadenectomy has been associated with an improved survival in a specific subset of patients with early stage [7,8] and node negative [9] gastric cancer. Although stage migration probably contributes to this improved survival, there may still be a small group of patients who may benefit from resection of micrometastatic disease.

Although IHC evaluation of lymph nodes has been used to rationalize the utility of an extended lymphadenectomy, it can also be used to argue against it. In most Western countries, lymph nodes are regarded as *indicators* rather than *governors* of outcome for gastric adenocarcinoma. According to this philosophy, extended lymph node dissection merely improves the accuracy of tumor staging [10]. The reluctance of surgeons in the West to use more radical resections is based on the lack of evidence that extended resections contribute to a survival benefit [11]. It is possible that IHC evaluation of D1 lymph nodes may obtain similar staging information as H&E evaluation of a D2 dissection. Because only 1 of the 25 patients in our series underwent an extended lymphadenectomy, we cannot address this point. However, in the reports by Izbicki et al. [6] and Natsugoe et al. [12], patients with esophageal cancer and IHC positive cells in their lymph nodes had similar outcomes to patients with histopathologically proven lymph node metastases. It is possible that a more intense examination of fewer lymph nodes offers the same prognostic and staging information as standard H&E evaluation of many lymph nodes provided by an extended lymphadenectomy.

CONCLUSIONS

In summary, our data indicate that the presence of IHC-detected lymph node micrometastases is associated with a worse prognosis for patients with histologic node negative gastric cancer. In addition, diffuse-type histology correlates with IHC positivity. IHC lymph node evaluation might be a useful staging modality in this subset of patients.

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